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Notch signaling reveals developmental plasticity of *Pax4*$^+$ pancreatic endocrine progenitors and shunts them to a duct fate

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Abstract

Relatively little is known about the developmental signals that specify the types and numbers of pancreatic cells. Previous studies suggested that Notch signaling in the pancreas inhibits differentiation and promotes the maintenance of progenitor cells, but it remains unclear whether Notch also controls cell fate choices as it does in other tissues. To study the impact of Notch in progenitors of the β cell lineage, we generated mice that express Cre-recombinase under control of the *Pax4* promoter. Lineage analysis of *Pax4*$^+$ cells demonstrates they are specified endocrine progenitors that contribute equally to four islet cell fates, contrary to expectations raised by the dispensable role of *Pax4* in the specification of the α and PP subtypes. In addition, we show that activation of Notch in *Pax4*$^+$ progenitors inhibits their differentiation into α and β endocrine cells and shunts them instead toward a duct fate. These observations reveal an unappreciated degree of developmental plasticity among early endocrine progenitors and raise the possibility that a bipotent duct-endocrine progenitor exists during development. Furthermore, the redirection of *Pax4*$^+$ cells from α and β endocrine fates toward a duct cell type suggests a positive role for Notch signaling in duct specification and is consistent with the more widely defined role for Notch in cell fate determination.

Keywords: Notch; *Pax4*; Endocrine; Duct; Pancreas development; Cell fate specification; Lineage; Cre-recombinase

1. Introduction

It is becoming clear that a detailed understanding of normal pancreatic development will be critical for the treatment of pancreatic diseases. Specifically, the directed differentiation of β cells in vitro for cell therapy purposes and the identification of processes that contribute to pancreatic cancers require more information about how pancreatic progenitors choose their cell fates during development. Two outstanding questions about the generation of cell fate diversity in the pancreas are the identity of the signals that promote the differentiation of mature cell fates and the nature of the progenitors that respond to those signals.

During embryogenesis, the pancreatic epithelium proliferates, branches and differentiates to produce three tissue types: exocrine cells that secrete digestive enzymes, ducts that form conduits to the duodenum, and endocrine cells that cluster to form the islets of Langerhans and release hormones into the bloodstream. Endocrine subtypes, including α, β, δ, and PP cells, differentiate sequentially and produce the hormones glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. Although little is known about the mechanisms that specify these cell fates, it has recently become possible to address this question using the Cre-loxP system in transgenic mice. Driver lines that express Cre-recombinase under different pancreatic promoters can be crossed to Cre-activated reporter and responder lines to characterize the fate and the potential of defined populations of progenitors (Branda and Dymecki, 2004).

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Notch signaling regulates the development of many tissues by controlling the time at which progenitors differentiate and by influencing their cell fate choices in a manner that is highly dependent on cell context (for reviews see Artavanis-Tsakonas et al., 1999; Raible and Eisen, 1995). Early in foregut development, Notch signaling in the endoderm has been shown to regulate Ptfla expression and define the normal boundaries of the pancreatic domain (Fukuda et al., 2006). Later, in the early pancreatic epithelium, Notch signaling is thought to promote the maintenance of a progenitor population at the expense of both exocrine and endocrine differentiation. Loss of Notch signaling in the pancreas of mouse and zebrafish embryos results in stunted growth and the precocious differentiation of either endocrine or exocrine cells (Apelqvist et al., 1999; Esni et al., 2004; Fujikura et al., 2006; Hald et al., 2003; Jensen et al., 2000; Lorent et al., 2004; Yee et al., 2005). Conversely, expression of the constitutively active intracellular domain, Notch-IC, traps pancreatic progenitors in an undifferentiated state and prevents the formation of endocrine and exocrine tissue (Esni et al., 2004; Lee et al., 2003; Lorent et al., 2004; Murtaugh et al., 2003). The fate of such Notch-expressing cells is to form tubules that express the early pancreatic marker, *Pdx1*. During normal development then, Notch signaling must be carefully regulated to balance the expansion of a progenitor pool with differentiation of mature cell types.

In many tissues, Notch regulates cell fate choices as well as the timing of differentiation. In the pancreas, it is possible that Notch signaling plays a positive role in duct specification since duct markers are lost from the early pancreas of zebrafish Notch mutants (Lorent et al., 2004; Yee et al., 2005). However, inactivation of Notch signaling in the early mouse pancreas by deletion of a floxed RBP-J_K allele using the Pdx1-Cre driver line appears to cause the formation of cystic tubules that express duct markers (Fujikura et al., 2006). This study suggests that, at the very least, Notch signaling is not required for duct differentiation. Although, no gain-of-function analyses support the hypothesis that Notch promotes the duct fate in the pancreas, the expression of Notch-IC in the embryonic zebrafish liver induces ectopic biliary ducts (Lorent et al., 2004), and the components of the Notch signaling pathway remain highly expressed in pancreatic epithelial tubules from which the ducts are likely to arise (Apelqvist et al., 1999; Lammert et al., 2000). In addition, the duct-like structures that form in malignancies such as pancreatic ductal adenocarcinoma exhibit high levels of Notch signaling (Miyamoto et al., 2003) and are of great interest in terms of their relationship to normal developmental processes.

Although Notch signaling inhibits the differentiation of early pancreatic progenitors, it is less clear what its effects may be on progenitors of intermediate maturity. In the mouse, it has been difficult to fully characterize the effect of Notch signaling in defined pancreatic progenitors due to lack of appropriate cell-specific driver lines. The expression of Notch-IC in *Ngn3*$^+$ endocrine progenitors has been shown to inhibit their differentiation (Murtaugh et al., 2003), but the ultimate fate of these cells could not be analyzed due to embryonic lethality, presumably due to Notch misexpression in the *Ngn3*$^+$ domain outside of the pancreas. By contrast, expression of Notch-IC in fully differentiated insulin$^+$ cells has no demonstrable effect, indicating that Notch function in the pancreas does indeed depend on cell context (Murtaugh et al., 2003).

One population of pancreatic cells that offer an important target for lineage analysis are progenitors that express the paired domain transcription factor, *Pax4*. Like *Ngn3*, *Pax4* is transiently expressed in endocrine progenitors during pancreatic development and is downregulated shortly after birth. However, the expression of *Pax4* is thought to be downstream of *Ngn3*, since *Pax4* expression is lost in *Ngn3* null mutants but not vice versa (Gradwohl et al., 2000; Wang et al., 2004). *Pax4*$^+$ cells are found in the undifferentiated pancreatic epithelium, which also contains undifferentiated duct and exocrine progenitors, and eventually delaminate and express hormones. Unlike *Ngn3*, which is widely expressed in the intestine and nervous system (Jenny et al., 2002; Lee et al., 2003), *Pax4* expression is largely restricted to the pancreas and duodenum making it a better candidate for driving ectopic expression in embryos (Larsson et al., 1998; Sosa-Pineda et al., 1997). In addition, the fate of *Pax4*$^+$ cells in normal development represents an interesting focus of inquiry since *Pax4* has been implicated in acquisition of endocrine subtype identity. In the absence of *Pax4*, β and δ cells fail to develop and more α cells are observed (Collombat et al., 2003, 2005; Sosa-Pineda et al., 1997). This reciprocal effect on subtype differentiation suggests that *Pax4* may normally function to bias endocrine progenitors away from the α fate toward β/δ fates.

To determine the fate of *Pax4*$^+$ progenitor cells under conditions of normal development and after expression of Notch-IC, we generated transgenic mice that express Cre-recombinase under control of the *Pax4* promoter. By lineage tracing with a heritable Cre-activated reporter, we find that *Pax4*$^+$ cells are specified endocrine progenitors that contribute equally to each of four endocrine subtypes during normal development. As a population then, *Pax4*$^+$ cells are not biased toward a particular subtype fate, even though the knockout phenotype suggested that they would preferentially adopt β/δ fates. Nonetheless, when *Pax4*$^+$ progenitors are forced to express activated Notch, some are shunted away from α and β fates and instead contribute to ducts. Thus, *Pax4*$^+$ cells have both a broader fate and a wider developmental potential than previously appreciated. Moreover, the ability of Notch to promote differentiation of the mature duct fate is unprecedented in the pancreas. It is, however, similar to the classically defined role for Notch in the choice between "primary" and "secondary" fates and the glial promoting effects of Notch in the nervous system (for reviews see Artavanis-Tsakonas et al., 1999; Raible and Eisen, 1995).